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VIRULENCE IN USTILAGO HORDEI (PERS.) LAGERH.

by

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A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Virulence in Ustilago hordei (Pers.) Lagerh." submitted by Percy L. Thomas in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

All possible crosses of the 12 meiotic products derived from three teliospores of Ustilago hordei (Pers.) Lagerh. were made and used to infect 13 varieties of barley. F_1 crosses from this material showed that a low level of virulence on the varieties Gateway and Olli was caused by a single recessive gene in the pathogen. A slightly higher level of virulence on Keystone was shown to be produced by another single recessive gene.

Segregation of factors causing different percentages of infection levels on Wolfe in one of the original parental spores was found. The cause of the variation was attributed to two dominant, interacting genes at separate loci.

A low percentage of infection was found when combinations of sporidia of non-compatible mating types were placed on the variety Odessa. This unexpected result was attributed to mutation of the mating type locus.

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Table of Contents

List of Tables

INTRODUCTION

The smut species Ustilago hordei (Pers.) Lagerh. is parasitic on barley. Cultures of this species may be separated into physiologic races, according to their parasitic interactions with a standard group of test varieties. The races thus defined need not be genetically pure and, as was pointed out by Nicoliasen (15), a field collection of smut teliospores is in reality a population, comprising many different genotypes. A study of individual genotypes within such populations would provide information on their composition and, at the same time, may provide some insight as to how the parasite may be controlled.

U. hordei is well suited to the genetic experimentation required to define the individuals in a population for the following reasons:

(i) sporidia (i.e. gametes) are formed in an ordered tetrad.
(ii) the sporidia can be multipled in vitro as vegetative clones.
(iii) controlled matings can be carried out to produce the diploid (dikaryotic) parasitic stage.

By proper manipulation of the correct sporidial lines, experimentation involving different aspects of virulence can be accomplished. The present study was undertaken in an attempt to elucidate some of these aspects, namely:

(i) the identification of the genetic factors responsible for virulent and avirulent host - parasite reactions.
(ii) the identification of the genetic factors responsible for variations in percentages of infection on specific host varieties.
(iii) an examination of the compatibility system of U. hordei.

LITERATURE REVIEW

Physiologic Race Studies

In 1924a Faris (9) demonstrated physiologic specialization in U. hordei by showing that five different races could be differentiated on suitable host varieties. Rodenhiser (17) later identified seven races by cultural characteristics. Two of these differed pathogenically on the varieties Lion and Himalaya.

Aamodt and Johnston (1) found evidence of two physiologic races in 1935. Semeniuk (19) placed twelve teliospore collections on four varieties and distinguished four races. Later tests with the same material gave conflicting results.

In 1937 Tapke (21) inoculated the varieties Excelsior, Hannchen, Lion, Nepal, Pannier, Gatami, Odessa and Trebi with 200 collections of teliospores of U. hordei from 26 states. His classification of races was based on resistant, intermediate and susceptible reactions (i.e. 0 - 5%, 6 - 35% and 36 - 100% infection, respectively). Tapke identified eight races, of which race six was the most prevalent. In 1945 Tapke (23) identified five additional races using the same differential varieties except for Gatami, which was replaced by Himalaya.

Cherewick (5) found all 13 races in Canada. He demonstrated that the addition of new differentials can increase the number of races defined. White Hulless and Newal added to Tapke varieties increased race definition from 13 to 16.

Genetic Studies

Controlled genetic studies of pathogenicity on a smut were first carried out by Nicolaisen (15) in 1934. These studies on Ustilago avenae (Pers.) Rostr. indicated that pathogenicity against the variety Gopher is dominant, against von Lochow is recessive, and against Lischower both dominant and recessive.

Holton and Halisky (12) concluded that each differential reaction exhibited by the races of covered smut, which they studied on U. avenae, was conditioned by a single recessive gene for virulence. In 1964 Holton (13), using inbred races and hybrid combinations between these races, showed that virulence was dominant on some varieties and recessive on others.

In an investigation on intra- and interspecific crosses in oat smuts (U. avenae and U. kolleri) and barley smuts (U. hordei and U. nigra) Cherewick (7) found that the genetic control of virulence may vary from recessive to incompletely dominant to dominant.

Nicolaisen (15) concluded that individual spores within a specific race population may be genetically heterozygous with respect to the factors controlling virulence on certain varieties. Cherewick (6) further illustrated the variability of race collections by placing the same race on the same host for several generations. The infectivity in one case involving U. avenae increased from 8% to 92% after eight generations of repeated culture on a single host. Holton (13) found genetic variability in individual spores taken from a specific race population.

Methods of Inoculation

Preparation of the barley seed for inoculation with U. hordei involves an effective method of either getting the inoculum under the hull, or removing the hull, without harming germination. Several investigators have attempted to develop effective techniques.

Tisdale (26), Briggs (4) and Aamodt and Johnston (1) found hand dehulling increased the percentage infection but decreased germination. Johnston (14) found that dehulling with sulfuric acid reduced germination and gave erratic results. Popp and Cherewick (16) devised a method for loosening barley hulls which employed a modified Waring Blendor.

In 1935 Tapke (20) shook spores in a water suspension and forced them under the barley hull with 30 inches of vacuum for 15 minutes. Higher infection percentages were obtained when the seed was pre-treated with a formaldehyde solution (1 to 320) for one hour followed by a wash in running water and air drying. The formaldehyde also served effectively to stop any latent infection from developing.

Holton (13) inoculated paired sporidial cultures by removing monosporidial lines from potato sucrose agar cultures and mixing them in water 12 to 24 hours before pouring them over the seeds. The combination was then placed in a vacuum.

Effect of Environment on Infection

The lack of consistency in obtaining high percentages of symptom expression can be attributed to:

(i) imperfect contact of the inoculum with the germinating seed.

(ii) the genetic constitutions of the host and pathogen.

(iii) variations in the environment around the seed and the plant after inoculation.

The published information on the relation of the environment to the cereal smuts has been reviewed by Tapke (24). Therefore, only selected papers dealing with environmental effects pertinent to the results of this thesis will be reviewed here.

Faris (10) has found that soil temperature during pre-emergence of the barley had an effect on the percentage infection. Schafer et al. (18) worked with a moderately resistant variety and a highly susceptible variety. By varying temperature they found that the pathogen favored a specific temperature range for maximum expression of symptoms. The basic nature of the resistant and susceptible reactions was not changed by the variation in temperature; it was merely shifted within a limited range.

Taylor and Zehner (25) reported that seed sown three inches deep showed more infection than did seed sown only one-half inch deep.

Wells (28) mentioned that seedling mortality in susceptible plants was greater than that found in resistant or moderately resistant plants when the plants were infected.

MATERIALS AND METHODS

Spores from the Edmonton and Winnipeg areas were collected. A number of these were germinated so that monogenetic sporidial lines could be established. Twelve sporidial lines, representing the haploid products of meiosis of three arbitrarily selected spores, were used as the parental material for all subsequent experimentation. Spore A represented the Winnipeg material while spores E and I were found at the University farm in Edmonton.

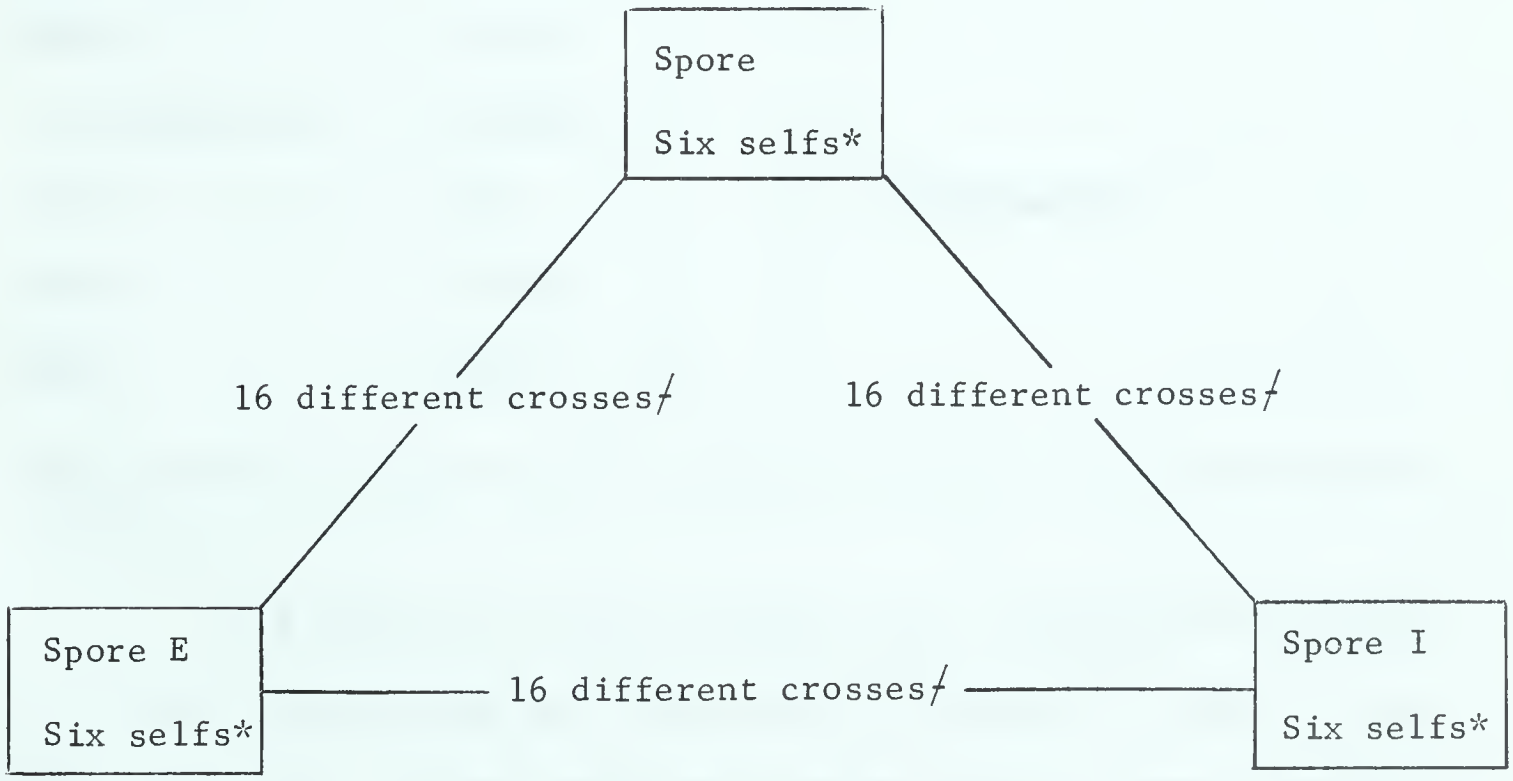
All culturing was carried out on the complete medium recommended by Vogel (27) for Neurospora crassa. Isolation of the four products of meiosis from the budding promycelium, and their subsequent transfer to new media to set up separate cultures, was done by standard procedure (8) using a de Fonbrune micromanipulator. Sporidia and the lines developing from them were numbered one to four, number one being the sporidium arising from the apex of the promycelium. Stock cultures were stored in test tubes on agar medium in a refrigerator at 2 to 5° C and sub-cultured every five to six months. Compatibility of the sporidial lines was determined by the Bauch test (2) (3), cited by Fischer and Holton (11), carried out on Vogel minimal medium. Sporidium number one of spore A was assigned (+) mating type and all other sporidial lines were assigned mating types on this basis.

Test number one was designed to give as much information as possible about the virulence of the sporidial lines so that this information could be used as a basis for future testing. The way to obtain complete information was to self and intercross the sporidial lines in

all possible combinations (Diagram number 1). Non-compatible types were also mated to test the validity of the results obtained from the Bauch tests. Also desirable was to place the inoculum on a wide range of host varieties. The eight differential varieties used by Tapke in 1945 (23) have well-known reactions to different races of U. hordei so these were incorporated into the test. Since it was also desirable to use common varieties, five of these were selected. Table I lists the varieties of barley used.

Diagram 1

Diagrammatic representation of the sporidial combinations placed on the 13 differential varieties of barley for test 1



* Crosses or sporidial combinations of the four sporidial lines from one spore.

/ All possible combinations, legitimate and illegitimate, of the four sporidial lines from each spore.

Table I. Barley varieties used as testing or differential varieties during the course of the experimentation

C.I. No.		
1248	Excelsior	Tapke, 1945
541	Hannchen	
620	Himalaya	
923	Lion	
595	Nepal	
182	Odessa	
1330	Pannier	
936	Trebi	
10072	Gateway	Supplemental
292 (C.A.N.)	Keystone	
6251	Olli	
7324	Vantage	
10071	Wolfe	

All barley seeds were treated with a formaldehyde solution (1 to 320) for one hour and then washed in running tap water for one half hour (22). The wet seeds were spread thinly on paper towels to air dry. The Waring Blendor treatment used by Popp and Cherewick (16) was used to further loosen the barley hulls and the seeds were again air dried on paper towels.

Sporidial lines were inoculated on fresh agar in test tubes and allowed to grow for four to five days. The actual crossing was accomplished by placing the fresh inoculum from these slants in the appropriate combinations into 125 ml. Erlenmeyer flasks containing 100 ml. of Vogel (complete) liquid medium. The flasks were stoppered with #4 rubber stoppers and placed on a shaker. The inoculum was multiplied in shake culture for a period of 24 to 36 hours. Sporidial counts taken from these cultures at this time ranged from six to nine million per milliliter.

The barley seeds were counted and placed in three dram vials. The sporidial inoculum was poured over the seeds and the vials were placed in a vacuum chamber. A pressure of 20 inches was applied for 20 minutes (22). Excess inoculum was poured from the seeds which were placed in small coin envelopes. The tops of the envelopes were left open so that the seeds could air dry. After a minimum of three days the seeds were sown.

Test number one was sown in pots in the University growth chambers in September, 1963. Fifteen seeds were placed 3/4" deep in a California mix soil in each six inch pot. Since 30 seeds were exposed to each sporidial combination two pots were required per cross. The pots were placed side by side on the benches with no spacing. Seeds were germinated at 65° F and grown at 75° F ($\pm$ 5° F due to available controls) using a ten hour day as suggested by Schafer and Dickson (18). During its growth the crop had to be treated with insecticide for aphids and sulphur dust for powdery mildew. These problems and the crowding of the pots and the seeds within the pots caused a number of

plants to die but did give sufficient data to suggest further lines of research.

Test number two was designed to verify the results obtained in test number one and also investigate some of these results further. One hundred seeds were used for every host - parasite combination so that the data would express a degree of statistical reliability. The 100 seeds were sown one inch deep in 24-foot rows enabling a two to three inch plant spacing. The rows were spaced one foot apart. The test was sown at the University farm in the spring of 1964.

The first part of test two involved a repeat of test one, omitting the non-compatible crosses and the three varieties (Excelsior, Nepal and Pannier) which were not infected.

The second part of test two was based on the results of test one on the varieties Gateway, Keystone and Olli. To investigate the genetic cause of these results a series of A x E and A x I F_1 hybrid spores were obtained from other barley varieties. The meiotic sporidial products of the four selected spores were selfed and intercrossed in all possible compatible combinations on the three barley varieties.

The final part of test two was designed to explain the variation in percentage infectivity of the selfed sporidial lines of Spore A on Wolfe, E on Odessa and I on Olli. Three F_1 spores from each of the four selfs of each spore were isolated. The 12 F_1 spores from Spore A were selfed in all possible compatible combinations on Wolfe, the 12 F_1 spores from E were selfed on Odessa, and the 12 F_1 spores from I were selfed on Olli.

Part one of test number three was designed to further investigate the results on Wolfe found in test two. Two F_2 spores were selected, germinated and selfed on Wolfe to obtain F_3 data.

The second part of test three was an investigation into some unexplained aspects of compatibility or mating types. A mistake in reading the Bauch tests on two of the F_1 spore lines used to infect Odessa in the final part of test two resulted in four incompatible crosses being made. These crosses yielded a low percentage infection. Four F_2 spores from these low infection groups were germinated and the meiotic products of each spore were selfed on Odessa in compatible and non-compatible combinations. The compatible crosses originally missed, due to improper sexing, were made in order to get complete results from the spores in question. The eight monosporidial lines of the two spores involved were inoculated separately on Odessa to check each line.

Test three was sown in the growth chambers in November of 1964. Ten seeds were sown 3/4" deep in California mix in each six inch pot. The pots were spaced to give the plants a less crowded growth environment. One hundred seeds were treated with each inoculation to give sufficient data. Seeds were germinated at 65^{o} F and grown at 75^{o} F $\pm$ 5^{o} F with a 16 hour day. Plants were treated with insecticide for aphids and sulphur dust for powdery mildew.

All counts of percentage infection for the three tests were based on the number of plants infected (i.e. if a plant with 25 tillers had one tiller holding a partially infected head the plant was counted as infected).

RESULTS

Reactions of the Three Parental Spores on the Differential Hosts

Test number one, in which the twelve sporidial products of the three original spores were crossed in all possible combinations, gave the following general results:

1. The varieties Excelsior, Nepal and Pannier were resistant to the sporidial lines used.

2. The non-compatible ++ and -- sporidial combinations gave no infection. This confirms the reliability of the Bauch tests and shows that the formaldehyde seed treatment was effective in removing the ability of any contaminant spore to infect the plants.

3. Spore A (when selfed) infected Keystone while E and I (when selfed, intercrossed and crossed with A) did not. Spores E and I (when selfed and intercrossed) infected Gateway and Olli but A selfs or crosses of E x A or I x A did not infect these varieties.

4. Symptom expression was low on the varieties Gateway, Keystone and Olli.

5. A variation existed in the percentage infectivity of the selfed sporidial lines of the individual spores. This could have been a result of the low population size or genetic segregation of virulence factors in the pathogen.

Test number one, when repeated in the field using larger populations, gave the results shown in Table II. Points not found in experiment number one are:

1. The E x E, I x I, A x E and A x I crosses on Keystone showed variants that gave low infection percentages.

2. Gateway and 011i exhibited a very low percentage infection when exposed to virulent sporidial combinations.

3. E x E and I x I sporidial combinations did not show the variability in symptom expression previously shown in test one. The A x A combinations retained their variability.

Recognition of Genes for Virulence on Gateway, 011i and Keystone

Part two of test two involved the selfing and intercrossing of A x E and A x I F_1 hybrids on the varieties Gateway, Keystone and 011i. The results of this test are shown in Table III. Spores 6-21 and 6-22 are A x E hybrids isolated from variety 6 (Odessa) exposed to crosses number 21 and 22 of test number one. Spores 6-42 and 6-49 are A x I hybrids isolated from Odessa exposed to crosses 42 and 49 of test one.

The results on Gateway and 011i were analyzed by assigning a homozygous recessive genotype (gg) to each of the relevant infection classes. This assignment of genotype to pathogenic cultures automatically enabled the determination of the genotypes of all other sporidial lines and thus of all sporidial combinations. The assumption that the same

Table II. Infection percentages resulting from crossing the sporidial lines from the three parental spores in all possible compatible combinations on the differential barley varieties

Sporidial combination		Hannchen	Himalaya	Lion	Odessa	Trebi	Gateway	Keystone	Olli	Vantage	Wolfe
	1x2	15	38	9	22	5	0	13	0	20	3
AxA	1x4	34	48	27	59	43	0	8	0	47	13
	2x3	13	30	9	62	32	0	2	0	22	5
	3x4	46	33	34	75	57	0	18	0	64	13
	1x3	56	0	30	78	53	0	1	1	74	9
ExE	1x4	66	2	30	*	*	*	0	2	69	12
	2x3	60	2	27	84	63	5	1	0	71	7
	2x4	56	0	31	86	51	3	0	2	70	8
	1x3	64	0	28	80	52	6	0	6	76	9
IxI	1x4	74	0	21	94	52	1	0	1	75	16
	2x3	76	2	23	84	41	1	0	0	64	13
	2x4	56	0	20	78	43	4	0	1	60	7
	1x3	30	0	22	69	39	0	0	0	51	14
	1x4	41	3	25	63	51	0	3	0	58	15
	2x1	26	0	21	59	34	0	0	0	56	8
AxE	2x2	42	2	9	63	24	0	0	0	27	6
	3x3	67	2	29	78	64	0	1	0	62	14
	3x4	70	6	40	80	66	0	0	0	71	24
	4x1	71	0	38	83	68	0	0	0	74	9
	4x2	60	0	34	81	53	0	0	0	73	19
	1x1	36	0	21	75	48	0	0	0	61	21
	1x2	41	0	17	70	44	0	0	0	56	5
	2x3	28	0	19	45	28	0	0	0	54	7
AxI	2x4	33	0	16	53	37	0	0	0	48	6
	3x1	69	0	32	76	56	0	0	0	72	33
	3x2	47	2	33	74	56	0	0	0	62	22
	4x3	75	2	39	79	54	0	1	0	80	34
	4x4	78	0	40	94	57	0	1	0	70	31
	1x1	68	0	49	80	60	8	0	3	74	9
	1x2	64	0	40	82	53	7	0	7	66	8
	2x1	49	0	24	89	35	2	0	3	62	10
ExI	2x2	61	0	17	78	42	3	0	4	57	11
	3x3	45	0	17	86	43	5	0	4	47	8
	3x4	71	0	25	74	36	4	0	3	69	8
	4x3	68	0	43	86	51	8	0	5	79	17
	4x4	40	0	43	81	55	3	0	0	76	18

* Accidentally lost while sowing.

Table III. F_2 results on three varieties

Mating type	Spore No.	Cross	% infection Gateway	Olli	Keystone	Pathogen genotype
1 -		1 x 2	0	0	1*	Gg Kk
2 +	6-21	1 x 3	9	20	0	gg Kk
3 +		2 x 4	0	0	1*	GG Kk
4 -		3 x 4	0	0	0	Gg Kk
1 -		1 x 2	3	5	0	gg Kk
2 +	6-22	1 x 4	0	0	0	Gg Kk
3 -		2 x 3	0	0	5*	Gg Kk
4 +		3 x 4	0	0	2*	GG Kk
1 -		1 x 3	0	0	1	Gg kk
2 -	6-42	1 x 4	4	2	0	gg Kk
3 +		2 x 3	0	0	0	GG Kk
4 +		2 x 4	0	0	0	Gg KK
1 -		1 x 2	0	0	0	Gg KK
2 +	6-49	1 x 3	0	0	2*	Gg Kk
3 +		2 x 4	0	1*	1*	Gg Kk
4 -		3 x 4	0	0	18	Gg kk

Cross		% infection Gateway	Olli	Keystone	Pathogen genotype
	1 x 2	17	24	0	gg Kk
	1 x 4	0	0	4*	Gg Kk
	2 x 1	0	0	1*	Gg Kk
6-21	2 x 3	0	0	0	GG Kk
x	3 x 1	3	10	1*	gg Kk
6-22	3 x 3	0	0	2*	Gg Kk
	4 x 2	0	0	0	Gg Kk
	4 x 4	0	0	0	GG Kk
	1 x 3	0	0	3	Gg kk
	1 x 4	2	1	0	gg Kk
	2 x 1	0	0	0	Gg Kk
6-21	2 x 2	0	0	0	GG KK
x	3 x 1	2	0/	0	gg Kk
6-42	3 x 2	0	0	0	Gg KK
	4 x 3	0	0	8	GG kk
	4 x 4	0	0	0	Gg Kk
	1 x 2	0	0	1*	Gg Kk
	1 x 3	0	1*	26	Gg kk
	2 x 1	0	0	0	Gg KK
6-21	2 x 4	0	0	0	Gg Kk
x	3 x 1	6	3	1*	gg KK
6-49	3 x 4	0/	0/	0	gg Kk
	4 x 2	0	0	0	GG Kk
	4 x 3	0	0	18	GG kk

/ Should be infected but failed to show symptoms.
* Variant expression of pathogenic symptoms.

Table III - continued

Cross		% infection Gateway	Olli	Keystone	Pathogen genotype
	1 x 3	0	0	7	Gg kk
	1 x 4	1	0/	0	gg Kk
	2 x 1	0/	3	1*	gg Kk
6-22	2 x 2	0	1*	0	Gg KK
x	3 x 3	0	0	6	GG kk
6-42	3 x 4	0	0	0	Gg Kk
	4 x 1	0	0	0	Gg Kk
	4 x 2	0	0	0	GG KK
	1 x 2	0	0	3*	Gg Kk
	1 x 3	0	0	34	Gg kk
	2 x 1	4	1	0	gg KK
6-22	2 x 4	8	8	0	gg Kk
x	3 x 2	0	0	0	GG Kk
6-49	3 x 3	0	0	14	GG kk
	4 x 1	0	0	0	Gg KK
	4 x 4	0	0	0	Gg Kk
	1 x 2	0	0	0	Gg Kk
	1 x 3	0	0	15	Gg kk
	2 x 2	0	0	0	GG KK
6-42	2 x 3	0	0	0	GG Kk
x	3 x 1	0	0	0	Gg Kk
6-49	3 x 4	0	0	10	Gg kk
	4 x 1	2	1	0	gg KK
	4 x 4	1	3	0	gg Kk

/ Should be infected but failed to show symptoms
* Variant expression of pathogenic symptoms.

genotype is responsible for infection on both varieties is substantiated in that the two varieties are closely related (i.e. Gateway is the result of a Newal x Olli backcrossed to Olli cross). The failure of some of the gg genotypes to express symptoms of infection may be attributed to the low level of virulence caused by this genotype.

The results on Keystone were analyzed by assigning a homozygous recessive genotype (kk) to the relatively high infection percentage classes. The remaining genotypes were then calculated and in all cases the homozygous recessive sporidial matings caused infection. Variations (i.e. infections existing where the genotypes were inappropriate) were assumed to be caused by variations in the genotype giving the host resistance.

Inheritance of the Factors causing Variation in Virulence on Wolfe

The final part of test two involved the variability found in the A x A, E x E and I x I sporidial combinations. Three F_1 spores from each compatible combination of sporidia in the three preceding selfed lines were selected for further study. Sporidial products of each of the selected spores were brought together in all compatible combinations, producing four selfed cultures in each case. The results of this work as shown in Tables IV and V plus the results of the first part of test two (Table II) failed to reveal any evidence of variability due to heterozygosity in two of the original spores (i.e. E and I) on the varieties used.

The results of the selfing of the twelve spores obtained from the F_1 of spore A are shown in Table VI. Since additional data did not lead to a satisfactory explanation it was decided, by selfing two F_2 spores in the greenhouse, to obtain additional information.

The explanation of the genotypes assigned to each infection class is based on a hypothesis involving two dominant, interacting genes

Table IV. F_2 results on Odessa obtained from the selfing of three spores from each F_1 infection class

Parental cross		% infection
E x E	1 x 3	78
	1 x 4	/
	2 x 3	84
	2 x 4	86

Spore No.	Sporidial cross	% infection
1	1 x 2	85
	1 x 3	78
	2 x 4	80
	3 x 4	86
2	1 x 3	85
	1 x 4	81
	2 x 3	86
	2 x 4	88
3	1 x 2	86
	1 x 4	92
	2 x 3	83
	3 x 4	78
4	1 x 2	80
	1 x 3	11*
	2 x 4	3*
	3 x 4	81
5	1 x 2	79
	1 x 3	89
	2 x 4	79
	3 x 4	85
6	1 x 2	82
	1 x 4	4*
	2 x 3	3*
	3 x 4	85
7	1 x 2	74
	1 x 4	85
	2 x 3	87
	3 x 4	85
8	1 x 3	81
	1 x 4	88
	2 x 3	73
	2 x 4	77
9	1 x 2	77
	1 x 3	73
	2 x 4	78
	3 x 4	85
10	1 x 2	86
	1 x 4	77
	2 x 3	73
	3 x 4	89
11	1 x 2	89
	1 x 3	83
	2 x 4	86
	3 x 4	91
12	1 x 2	76
	1 x 3	80
	2 x 4	86
	3 x 4	69

/ Plot lost while sowing.
* Incompatible crosses;
see page 22.

Table V. F_2 results on Olli obtained from the selfing of three spores from each F_1 infection class

Parental cross		% infection
I x I	1 x 3	6
	1 x 4	1
	2 x 3	0
	2 x 4	1

Spore No.	Sporidial cross	% infection
1	1 x 2	4
	1 x 4	2
	2 x 3	7
	3 x 4	10
2	1 x 3	1
	1 x 4	7
	2 x 3	3
	2 x 4	2
3	1 x 3	1
	1 x 4	4
	2 x 3	2
	2 x 4	3
4	1 x 2	0
	1 x 3	2
	2 x 4	4
	3 x 4	1
5	1 x 2	4
	1 x 4	5
	2 x 3	4
	3 x 4	3
6	1 x 2	0
	1 x 4	2
	2 x 3	3
	3 x 4	1
7	1 x 2	3
	1 x 4	4
	2 x 3	2
	2 x 4	0
8	1 x 3	0
	1 x 4	1
	2 x 3	2
	2 x 4	0
9	1 x 3	1
	1 x 4	4
	2 x 3	2
	2 x 4	1
10	1 x 2	0
	1 x 3	1
	2 x 4	4
	3 x 4	4
11	1 x 2	2
	1 x 3	5
	2 x 4	5
	3 x 4	1
12	1 x 2	0
	1 x 3	0
	2 x 4	3
	3 x 4	1

Table VI. F_2 results, including predicted genotypes, on Wolfe obtained from the selfing of three spores from each F_1 infection class

A x A parental cross	% infection	Pathogen genotype	Sporidial combination	% infection	Pathogen genotype	F_3 data		
1 x 2	3	AaBB	1 x 2	15	aaBB	1x3	16	aaBB
			1 x 3	3	AaBB	1x4	4	aaBB
			2 x 4	5	AaBB	2x3	19	aaBB
			3 x 4	0	AABB	2x4	6	aaBB
			1 x 2	18	aaBB			
			1 x 3	1	AaBB			
			2 x 4	3	AaBB			
			3 x 4	0	AABB			
			1 x 2	0	AABB	1x3	2	AaBB
			1 x 3	8	AaBB	1x4	7	AaBB
			2 x 4	2	AaBB	2x3	6	AaBB
			3 x 4	16	aaBB	2x4	5	AaBB
1 x 4	13	AABb	1 x 2	22	AABb			
			1 x 3	14	AAbb			
			2 x 3	0	AABB			
			3 x 4	17	AABb			
			1 x 2	21	AABb			
			1 x 4	26	AAbb			
			2 x 3	0	AABB			
			2 x 4	0	Incompatible			
			1 x 3	16	AAbb			
			1 x 4	23	AABb			
			2 x 3	21	AABb			
			2 x 4	0	AABB			
2 x 3	5	aaBb	1 x 2	13	aaBb			aaBb
			1 x 4	15	aaBb			aabb
			2 x 3	20	aaBb			aaBB
			3 x 4	14	aaBb			aaBb
			1 x 2	15	aaBb			aaBB
			1 x 3	14	aaBb			aaBb
			2 x 4	9	aaBb			aaBb
			3 x 4	8	aaBb			aabb
			1 x 2	13	aaBb			aaBB
			1 x 4	12	aaBb			aaBb
			2 x 3	12	aaBb			aaBb
			3 x 4	10	aaBb			aabb
3 x 4	13	Aabb	1 x 2	10	Aabb			aabb
			1 x 4	24	Aabb			Aabb
			2 x 3	23	Aabb			Aabb
			3 x 4	30	Aabb			AAbb
			1 x 2	27	Aabb			
			1 x 4	31	Aabb			
			2 x 3	24	Aabb			
			3 x 4	33	Aabb			
			1 x 2	36	Aabb			
			1 x 3	28	Aabb			
			2 x 4	25	Aabb			
			3 x 4	26	Aabb			

Possible alternative genetic segregation

at two loci. Allele A is designated as giving a relatively high percentage infection while allele B promotes medium percentage infection. A necessary part of this explanation is that, through genetic interaction, genotype AABB is avirulent. Table VII presents the reactions expected of the various possible genotypes.

Table VII. Hypothetical genotypes and their predicted phenotypes on the variety Wolfe

Genotype	Symptom expression expected
AABB	0%
AABb	Medium high
AAbb	High
AaBB	Low
AaBb	0%?
Aabb	High
aaBB	Medium
aaBb	Medium
aabb	0%? 10%?

Individuals of genotype AaBb do not exist in the predicted segregation types so that the phenotypic expression assigned is not definite. Individuals of genotype aabb may exist in this test (see optional segregation of F_2 in Table VI). If segregation did occur then the aabb genotype results in a medium infection percentage. This could be due either to lack of resistance in the host or to the effect of another gene in the absence of either A or B.

Incongruent Inheritance of the Mating Type Locus on Odessa

Test number three was concerned with the compatibility system in U. hordei. A mistake in reading Bauch tests had resulted in the placing of incompatible sporidial combinations on the variety Odessa. These incompatible crosses unexpectedly gave low infection percentages. The relevant data, taken from Table IV, are presented again in Table VIII.

Table VIII. Excerpt from Table IV showing incongruent inheritance of the mating type locus on Odessa

	Compatibility types	Sporidial combination used	% infection
Spore #4	1 -	1 x 2	80
	2 +	1 x 3	11
	3 -	2 x 4	3
	4 +	3 x 4	81
Spore #6	1 -	1 x 2	82
	2 +	1 x 4	4
	3 +	2 x 3	3
	4 -	3 x 4	85

The first test involved in the investigation of this phenomenon was to carry out the crosses that had been inadvertently overlooked. The results are shown in Table IX. The percentage infection figures are slightly lower than those found in the field, possibly due to the environmental conditions found in the growth chambers (infection percentages were generally lower in the growth chambers).

Table IX. Test of remaining compatible genotypes

	Sporidial combination	% infection
Spore #4	1 x 4	53
	2 x 3	65
Spore #6	1 x 3	52
	2 x 4	62

The results indicate that these are indeed legitimate + - matings and not illegitimate ++ and -- combinations.

A check of the individual sporidial lines was accomplished by inoculating the lines singly on Odessa. The results are shown in Table X.

Table X. Tests of single, haploid lines on the variety Odessa

	Sporidial line	% infection
Spore #4	1	0
	2	0
	3	1
	4	1
Spore #6	1	0
	2	0
	3	0
	4	1

Controls utilizing uninoculated Odessa exhibited no infection.

Spores were isolated from each of the low infection classes in Table VIII. One spore from each class was selfed in all possible combinations. Bauch tests indicated simple + and - reactions on these sporidial lines. The results of these sporidial combinations are shown in Table XI. These results show that the incompatible ++ and -- combinations continue to exhibit low infection percentages.

Table XI. The infection percentages of legitimate and illegitimate combinations derived from four spores

Mating type combinations	% infection			
Spore #	1	2	3	4
+ +	2.6	3.4	1.2	2.4
+ -	71	48	53	62
+ -	60	69	54	70
+ -	68	75	55	63
+ -	73	68	53	60
- -	1.2	2.2	1.7	6.7

DISCUSSION

The most interesting aspect to arise from the experimentation was in the identification of a single gene governing a very low level of virulence on the varieties Olli and Gateway. Infectivity at the low levels recorded in these segregations are usually not noticed and, if they are, they are not usually accorded any significance. The recognition of this low level of virulence would be of great assistance when considering a physiologic-race population which has a low level of virulence on a particular variety, especially if the variety is being considered as an introduction to a new area. If the low level of virulence is caused by a gene with a high frequency in the population, the level of virulence on the variety will not increase until a new genetic factor for higher virulence is introduced and selected for. However, the gene for a low level of virulence will allow the race population to persist and, ultimately to provide (either through recombination or new mutation) the more virulent genotypes that will be selected out by the host. If, however, the low level of virulence is caused by a highly virulent gene of low frequency in the population, the level of virulence will rapidly climb due to selection by the host. Therefore the genetic examination of the low levels of virulence in a pathogenic race is of utmost importance if a new variety is to be released in the area inhabited by the race.

A convenient method of deciding whether the low virulence is due to the presence throughout the population of genes of low virulence or, alternatively, to the presence (in only a few individuals) of genes

for high virulence would be to screen the culture for two or three cycles on the variety in question. Cherewick's data (6) shows that host selection for high levels of virulence can be achieved in as few as three generations.

The results on Olli which vary from the expected when the proposed genotype is applied can be attributed to the fact that Olli has never been selected for resistance to covered smut. This means that the variety may be genetically heterogeneous with respect to covered smut resistance. To test this assumption it would be necessary to demonstrate that both susceptible and resistant plants are present within the variety. This could be carried out by harvesting healthy tillers from infected plants which were exposed to avirulent sporidial combinations. The seeds from these tillers, when sown after being inoculated with an avirulent sporidial combination, should show a relatively high percentage infection.

The results on the variety Keystone deviated to a greater extent than did those on Olli. The variety is described as having moderate resistance to covered smut infection. The assumption, made by the plant breeder, is that this resistance level is due to a polygenic resistance system which could quite possibley segregate to give less resistant plants. This hypothesis could be checked in the same manner as that proposed for Olli.

The genetic purity of any cereal variety is never precisely known. The variety is usually the result of selection, exerted on some but not necessarily all loci, following which some of the selected lines

are bulked to form the new variety. There is little doubt that the selection process continues even after the variety is commercially grown. The seed of the varieties Olli and Keystone was obtained from the seed stocks of the University of Alberta and therefore should be relatively "pure." The fact that an occasional plant or genotype may appear to deviate from normal is an unavoidable condition of the experiment.

The system of two dominant, interacting genes involved in the production of virulence at different levels of infection on Wolfe is an interesting one which should be tested further. Experimentation involving larger populations of plants, replication for statistical analysis, and controlled crosses is necessary. The crosses could be designed in such a manner as to give predictable results to confirm the proposed hypothesis. The genotypes AaBb and aabb could be obtained from the controlled crosses to further verify or disprove the predictions.

The hypothesis, if correct, illustrates that individual spores may be heterozygous at several virulence loci, and may therefore segregate many different genotypes. This means that the race from which the spore originated has an excellent survival or adaptive potential when faced with new varieties with different resistance factors.

The compatibility results on Odessa are tentatively explained in terms of mutation at the mating locus, leading to the occurrence of two different mating types in an originally homogeneous sporidial culture. The frequency of the new, mutated form within the culture, and therefore the relative frequencies of infective dikaryons, would be related to such factors as the rate at which the new mutation occurs

and the relative reproductive rates of the original and the mutant genotypes. The hypothesis obviously requires further supporting data. Nicolaisen (15) reported a similar finding in U. avenae but offered no explanation and, to the present time, no further note of this phenomenon has been found in the literature.

Throughout the study it was noted that when infection percentages were high, the total number of plants was reduced. Although this confirms an earlier report (28), the differences that were segregating were sufficiently large that the reduction in survival had no significant effect on the interpretations.

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